

Accelerated Breeding of Conifers with Emphasis on Pinaceae

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ACCELERATED BREEDING OF CONIFERS WITH EMPHASIS ON *PINACEAE*

INTRODUCTION

The purpose of this paper is to introduce the reader to techniques which may be used for accelerating seedling growth and flower induction. Interest in these techniques stems from a desire to reduce the length of the breeding cycle, i.e., to accelerate breeding and to thereby improve the efficiency of conifer tree improvement programs. The emphasis here will be on species of the *Pinaceae* family, as most of such work has been applied to this group.

The purpose of accelerating growth is to produce large, vigorous seedlings of uniform quality in a relatively short time. Some of the aspects of breeding programs which may thereby be improved include studies of genetic variation, seed orchard establishment and management, and vegetative propagation of improved stock. Stimulating precocious and enhanced flowering also serves to streamline breeding programs by reducing the time required to derive seed of desired crosses. There are several techniques which may be used to enhance flowering. These may be broadly delineated as follows: hormonal treatments (e.g., the application of gibberellins) and cultural treatments (e.g., root pruning, heat treatment, water stress, fertilization).

An important component of a floral induction program is indoor breeding culture. Growing trees in a breeding hall for all or part of the growing season facilitates the application of certain cultural treatments which are not feasible on material grown outdoors.

This review will examine four components of accelerated breeding:

- 1) indoor breeding culture - the breeding hall and some aspects of growing trees indoors;
- 2) accelerating growth - specifically, accelerating early seedling growth, some of the treatments and applications;
- 3) flower enhancement treatments - the use of gibberellins and adjunct cultural treatments; and,
- 4) natural sources of variation in flowering - the effects of genotype, age, and scion-rootstock interaction on flowering.

This paper will serve as a basis for the development and implementation of a breeding hall program. As such, it does not delve into early genetic testing, another potential component of accelerated breeding programs.

INDOOR BREEDING CULTURE

Indoor breeding culture refers to the practice of growing trees under controlled conditions, for all or part of a growing season for breeding purposes. While it is possible to apply certain growth and floral stimulation techniques outdoors, the use of a breeding hall affords much greater opportunity to manipulate plant growth and development. Furthermore, several of the more effective growth and induction treatments do necessitate the controlled environment provided by indoor culture.

The Breeding Hall

A breeding hall is a greenhouse-type structure utilized for the purpose of indoor breeding culture. Terms synonymous with the term breeding hall include breeding greenhouse, induction hall, maxi-hall, and maxi-haus. Conventional greenhouse structures are typically utilized as breeding halls. Special consideration, however, should be given to the type and size of structure used.

Structural Dimensions

Trees used for indoor breeding culture may be grown for several years and may reach considerable heights. The hall must therefore be of adequate size to accommodate whatever final tree height is expected. This will vary with the length of the breeding cycle, species, genotype, and cultural practices used to stimulate growth and flowering. For example, Karki (1976) studied the growth of several tree genera within the same breeding hall under a 24-hour photoperiod and optimum growth conditions (including CO₂ enrichment). He observed a wide range in height (Table 1).

Table 1. Height growth of several tree genera in response to continuous light and optimum growth conditions. Heights are presented for two ages, 84 days and 251 days.

Species	Height	
	84 days	251 days
Birch (<i>Betula</i> L.)	233 cm	7 - 9 m
Alder (<i>Alnus</i> B. Ehrh.)	175 cm	5 - 8 m
Larch (<i>Larix</i> Mill.)	35 cm	205 cm
Norway spruce (<i>Picea abies</i> (L.) Karst.)	26 cm	143 cm
Scots pine (<i>Pinus sylvestris</i> L.)	27 cm	96 cm

Source: Karki (1976).

In determining the amount of floor space required in the hall, the following points need to be addressed: 1) the number of clones in the breeding program; 2) the number of ramets per clone; 3) the floor space required for aisles, access, and workspace, and 4) the floor space required by each tree. This latter point depends largely on the same factors that determine minimum height requirements (i.e., length of breeding cycle, species, genotype, growing regimes, and induction techniques). In the indoor breeding of potted loblolly pine (*Pinus taeda* L.) grafts, for example, O'Gwynn (1983a) noted that two- and three-year-old trees required a gross spatial allocation (including access and aisles) of about 1.8 m² per tree, while those four and five years old required up to 3.7 m².

Plastic greenhouses have been used as breeding halls in Finland since the 1960's (Lapisto 1973). These are large semicircular structures of a ground-to-ground arch design which lack straight sidewalls. This type of structure is not ideally suited for breeding since the sloping sides could preclude the use of the full width of the hall. Smaller trees may, however, be used to effectively utilize this side space (see Karki 1979). Greenhouses of a wide range of sizes have been used for breeding purposes (Table 2).

Table 2. Examples of dimensions of greenhouses used as breeding halls

Length (m)	Width (m)	Height (m)	Floor area (sq m)	Source
10	6	3.5	60	Karki (1976)
45	16	6.0	720	Lepisto (1973)
100	16	6.0	1600	Karki (1979)
50	20	7.5	1000	Kleinschmit et al. (1980)
100	20	7.5	2000	Lepisto (1973)

In several regions of Ontario, gutter-connected greenhouses are now being used as breeding halls. These structures measure 5.5 m in width (per bay) and approximately 30.5 m in length (length may vary by 1.2 m increments). They have 3.1 m high side walls of acrylic and are topped by an arched roof of double polyethylene film. Two marked advantages to using this type of structure are the ability to use its full width, and to subsequently add on bays, in a side-by-side manner.

Environmental Control Systems

Several of the techniques used to accelerate growth or induce flowering depend on subjecting whole trees to special environmental conditions, such as increasing the photoperiod beyond that of natural day length or increasing CO₂ concentration above ambient levels. Many of these techniques are based on devices and technologies that are common, or at least easily applied, in today's greenhouses. Some of the following systems can be considered:

- 1) heating system - to maintain minimum temperatures within the hall during winter months. This is necessary for accelerated growth and certain flower induction treatments.

- 2) ventilation and cooling system - to maintain a ceiling on maximum temperatures during the summer months. This will reduce the possibility of heat stress, excessive transpiration and subsequent moisture stress.
- 3) lighting system - to extend the photoperiod beyond that of the natural day length. This is necessary for accelerated seedling growth.
- 4) irrigation system - for controlled application of water (and soluble fertilizers if applicable) to each tree or group of trees. Hand watering can be used on a small scale; a drip irrigation system can be used for larger scale operations. Precisely controlled water application and monitoring of internal moisture stress is required when utilizing water stress treatments.
- 5) carbon dioxide generators - to enrich the CO₂ content within the greenhouse above that of ambient levels, for the purpose of enhancing plant growth.

Container Versus Ground Beds

For the purpose of indoor breeding, trees may be grown directly in ground beds or in containers of an appropriate size and dimension. Advantages of the former include a low initial investment and lower operating costs. The use of containers, however, provides for much better control of induction and cultural treatments, and more flexibility in the handling of trees (Hanover 1977, Ross et al. 1985a). This may be important if, for example, significant variation in tree size results in different rates of water loss. Separate water and fertilizer prescriptions for each tree would not likely be feasible, but trees of similar size could be grouped together and the optimum prescription for that size applied to the group. This would not be possible with trees in ground beds, where the best average prescription applied to all trees would likely be the only feasible alternative.

Container-grown trees may also be shifted within the hall to more effectively utilize available growing space. This applies to any hall with an arched roof, but particularly to those of a ground-to-ground arch design. As they increase in height, trees may be "rotated" from areas of minimum height (sides of hall) to areas of greater height (the center). Potted trees can also be grown outdoors and shifted into and out of the hall as treatment regimes and breeding plans require. In this way, the hall might be effectively used for more than a single crop of trees.

Orchard Establishment and Management

For any breeding orchard, the selection of scion and rootstock are important considerations. This is especially true of indoor breeding orchards, owing to the relatively high costs and intensive cultural practices involved. The rootstock should be healthy, vigorous, and disease free to ensure rapid growth of the graft (Weir 1983). The combination of scion and rootstock used may also have a profound effect on the survival, growth and flowering of the graft.

In the case of a potted orchard, it is necessary to determine the adequate pot size and type, and the type of potting medium to be used. Greenwood et al. (1979) and O'Gwynn (1983a) recommended potting rootstock in 8- to 11-litre containers. In these cases, grafting was done indoors in late winter to early spring and established grafts were transplanted into larger pots during the first growing season. O'Gwynn (1983a) reported that 75-litre galvanized steel pots were sufficient to carry fast-growing loblolly pine through a six-year greenhouse breeding cycle. The maximum necessary pot size will be less for slower growing northern conifers, especially where cultural practices limit root growth. For example, Ross (pers. comm. 1985) effectively maintained Engelmann spruce (*Picea engelmannii* Parry) and white spruce (*Picea glauca* [Moench] Voss) grafts in 8-litre containers for the first three years, and then in 11-litre containers for another three to four years.

A potting medium mixture of 2:1:1 sand:peatmoss:vermiculite was found to support good growth of loblolly pine while permitting good drainage (Greenwood et al. 1979). Decomposed hogfuel (mill waste, ground bark/wood) has also proven to be an economical and effective potting medium for trees (Ross per. comm. 1985).

The use of pots necessitates steps to prevent roots from growing through the drain holes and into the ground. This may be done by either raising the pots on pallets, covering drainage holes with wire mesh, or periodically cutting the roots that grow through the holes (Weir 1983).

Other factors that need to be addressed in maintaining an indoor orchard will include: 1) fertilization - the types of fertilizer and regimes to be used; 2) irrigation - the irrigation system, watering regime and water quality; and 3) pest control. The importance of the proper application of the appropriate management system cannot be overstressed. As pointed out by Ross and Pharis (1982), potted trees will not tolerate mismanagement. They note that most of the problems encountered in indoor breeding culture are founded in a lack of knowledge or improper application of cultural treatments or both.

ACCELERATING GROWTH

Tree growth is marked by two distinct phases: a juvenile phase and a mature phase. The juvenile phase is generally, though not necessarily, distinguished as the initial period of growth during which a tree lacks the ability to flower (Sweet 1975). Consequently, this phase decidedly limits the efficiency of conventional tree breeding programs. However, the time required to reach the juvenile-mature phase change may be reduced by accelerating early seedling growth. Thereby the benefits afforded by the mature growth phase may be more quickly realized.

The key to accelerating the early growth of northern temperate tree species is to prevent the onset of dormancy and the concomitant cessation of height growth. This response is a trait of adaptive significance in that it ensures that trees are in the appropriate physiological state required to survive the long cold, winters typical of the north. Unfortunately, this trait results in a pattern of annual cyclic growth; for most of the year trees actually exhibit no shoot growth at all (Hanover et al. 1976).

The control of dormancy is mediated through the phytochrome; specifically, by its continual reversion into one of two forms (P₆₆₀ and P₇₃₀) in response to photoperiod. As seedlings mature, endogenous control factors may take over to regulate growth independently of photoperiod (Hanover 1980). Nevertheless, it is possible to accelerate the growth of seedlings of many coniferous species by extending the photoperiod with artificial lighting. Relatively low light intensities are required to prevent the onset of dormancy (e.g., 50 foot-candles (ft-c)), given that a minimum photoperiod (which will vary with species and genotype) is provided. To promote normal or enhanced growth rates, however, higher light intensities are necessary; Hanover (1976) suggests that 800 to 1000 ft-c, as provided by fluorescent lights or mercury vapour lamps, is suitable for most species. Typically, supplemental lighting is used only to extend the photoperiod, not to add to the intensity of natural daylight.

Although light intensity and duration are likely the most important factors in accelerating seedling growth, other factors should also be optimized to ensure that seedling growth is not limited under accelerated growth conditions. In discussing the "accelerated-optimal-growth" concept, Hanover (1976, 1980) and Hanover and Bongarten (1977), outline the many additional factors that need to be considered: 1) temperature, 2) mineral nutrients, 3) water, 4) carbon dioxide, 5) growth regulators, 6) mycorrhizae, 7) growing medium, 8) competition, and 9) pests.

Work with several tree species has shown that seedlings can be induced to maintain a continuous, free-growth state, given the appropriate lighting requirements and optimal growing conditions. For example, Hanover and Reicosky (1972) elicited a continuous growth response from seedlings of blue spruce (*Picea pungens* Engelm.) and Engelmann spruce by applying a 24-hour photoperiod (450 ft-c supplemental lighting) and optimal temperature, water and nutrient conditions over a six-month period.

Similarly, Young and Hanover (1976) grew white spruce and blue spruce from seed under a 24-hour photoperiod for six months. They observed that seedlings 2 years old or less exhibited free, indefinite shoot growth, apparently "... limited only by environmental restraints and not by internal control." These seedlings flowered at 4 years of age, and had a more rapid growth rate after outplanting, than controls. Seedlings that were 3 years old or more, however, had a single flush of growth and set bud. This suggests that vegetatively propagated, physiologically older, material may not respond well to accelerated growth conditions (Hanover 1977).

Wheeler et al. (1982), working with lodgepole pine (*Pinus contorta* Dougl.) grew both seedlings and mature grafted scions under continuous light (supplemental light intensity of 600 to 800 ft-c) for six months and subsequently outplanted them. Treated seedlings flowered by the third year from seed, producing "impressive" pollen and seed cone crops by the fifth year. The flowering response of treated grafts was substantially less than that of the seedlings. Treated seedlings and grafts both exhibited

significantly greater height growth than controls, though the response of treated seedlings was much greater than that of the grafts. The survival of treated grafts was 35% greater than that of controls (Wheeler 1979, Wheeler et al. 1982).

Wheeler (1979) noted that seedlings and grafts of lodgepole pine set bud and flushed repeatedly during the period of continuous photoperiod, a pattern also observed in other pines in response to similar treatment (see references, Wheeler 1979). This contrasts the response of some other conifer seedlings which exhibit a continuous, free-growth characteristic in response to extended photoperiod as, for example, blue spruce and Englemann spruce (Hanover and Reicosky 1972).

Apparently, many other species will respond to accelerated growth conditions. Hanover et al. (1976) present a comprehensive list of woody plant species that may respond to increases in photoperiod. Among the *Pinaceae*, this includes species of *Picea*, *Pinus*, *Larix*, *Abies* and *Pseudotsuga*. Hanover (1977) noted that of 40 to 50 woody species tested, all responded to supplemental lighting and other growth factors.

The increases in growth and early flowering associated with accelerated growth treatment provide means of improving several components of tree breeding programs. Hanover et al. (1976) discussed several advantages of the "accelerated-optimal-growth" system for tree production. This system produces larger seedlings of better, more uniform quality, which are subject to better growth and survival after outplanting than seedlings produced by conventional nursery practice. Seedlings are produced in months rather than years, facilitating most aspects of nursery management. Furthermore, accelerated growth may provide for more rapid and efficient genetic testing. Early, enhanced, and more regular flowering will reduce the time to produce and test desired crosses and make cross pollination work more efficient.

FLOWER ENHANCEMENT TREATMENTS

Several techniques have been developed to promote and enhance flowering in coniferous species. A distinction may be made between those treatments which are applied for the purpose of promoting flowering in juvenile, non-flowering material, and those which are aimed at enhancing the level and regularity of flowering in mature stock. This distinction depends more on the desired goals than on the actual treatments, since many flower enhancement techniques may do both when applied properly (Pharis 1976).

Common flower induction treatments may be broadly categorized as being either 1) hormonal or chemical treatments, or 2) cultural treatments. Into the former category fall a group of particularly effective hormones called gibberellins. The latter category includes water stress, root-pruning, heat treatment, out-of-phase dormancy, and fertilization.

Gibberellins

Much of the work done in the area of flower induction has dealt with the use of hormones, specifically gibberellins. Gibberellins have been linked with several aspects of plant growth (Pharis and Kuo 1977). The most common forms used in studies of flower induction are GA₃, GA₄, GA₅, GA₇, and GA₉; all are found occurring naturally in higher plants. For extensive reviews on the structure and effects of gibberellins on forest trees see Pharis (1975, 1976, 1977), Pharis and Kuo (1977), and Ross et al. (1983).

Gibberellins are of interest for two reasons: 1) they may promote flowering when applied exogenously, and 2) some cultural treatments that enhance flowering may also cause a concomitant increase in levels of endogenous gibberellins (e.g., Chalupka et al. 1982, Dunberg et al. 1983).

There are a number of variables to be considered in the use of gibberellin, several of which may have a profound effect on the outcome of the treatment.

These include the following:

- 1) the type of gibberellin used (i.e., GA₃, GA₄, GA₅, GA₇, GA₉);
- 2) the method of application (e.g., foliar spray application, topical application, stem or branch injection);
- 3) the concentration of gibberellin; and
- 4) the timing and duration of the application.

Type of Gibberellin

The polar gibberellin A₃ has been used effectively for flower induction with members of the *Taxodiaceae* and *Cupressaceae* for some time (see references in Pharis and Kuo 1977, Owens and Blake 1985). In contrast, GA₃ has been relatively ineffective with some genera of *Pinaceae* (e.g., *Pinus*, *Larix*, *Picea*, *Tsuga* and *Pseudotsuga*). For species of these genera, however, much success has been realized with the application of the less polar gibberellins A₄ and A₇, and in particular, with a mixture of the two, GA_{4/7}. As a result, most of the current work with gibberellins, as they may affect flowering in *Pinaceae*, has focused on GA_{4/7}. To date, GA_{4/7} has been used for flower induction with varying degrees of success on many species of *Pinaceae* including the following:

<i>Picea abies</i> (L.) Karst.	(Dunberg 1980)
<i>Picea glauca</i> (Moench) Voss	(Cecich 1985; Ho 1985; Marquard and Hanover 1985)
<i>Picea sitchensis</i> (Bong.) Carr	(Tompsett and Fletcher 1979; Philipson 1985)
<i>Picea engelmannii</i> (Parry)	(Ross 1985)
<i>Pinus taeda</i> L.	(Ross and Greenwood 1979; Greenwood 1982; Hare 1984)

<i>Pinus banksiana</i> Lamb.	(Cecich 1983)
<i>Pinus contorta</i> Dougl.	(Wheeler et al. 1980)
<i>Pinus sylvestris</i> L.	(Chalupka 1978, 1980, 1981)
<i>Pinus elliottii</i> Engelm	(Hare 1984)
<i>Pinus palustris</i> Mill.	(Hare 1984)
<i>Pseudotsuga menziesii</i> (Mirb.) Franco	(Pharis et al. 1980)
<i>Larix leptolepis</i> (Sieb and Zucc.) Gord.	(Bonnet-Masimbert 1982)
<i>Larix decidua</i> Mill.	(Bonnet-Masimbert 1982)
<i>Tsuga heterophylla</i> (Raf.) Sarg.	(Brix and Portlock 1982; Pollard and Portlock 1984)

Method of Application

Gibberellin is commercially available in a powder or tablet form. For the purpose of application, gibberellin is dissolved in ethanol and diluted with distilled water. One of three techniques may be used to apply the GA solution:

- 1) spray application directly onto foliage (e.g., Chalupka 1978, 1980, 1981; Ross et al. 1981; Cecich 1983, 1985; Marquard and Hanover 1984a, 1984b, 1985; Pollard and Portlock 1984);
- 2) topical application directly onto a branch or bud (e.g., Ross and Greenwood 1979; Luukkanen and Johansson 1980; Pharis et al. 1980; Wheeler et al. 1980; Greenwood 1981; Hare 1984); and
- 3) injection directly into the stem or a branch (e.g., Dunberg 1980; Ross et al. 1981; Puritch and McMullan 1981; Greenwood 1982; Philipson 1985).

The formulation of the GA solution varies with these three techniques.

Spray application - Preparation of the GA solution for spray application encompasses the following steps: a specific weight of GA is dissolved in ethanol. Distilled water is then added to bring the solution to

the desired concentration. One of three surfactants is added in desired proportions: 1) Aromox C-12/W* at 0.04 to 0.10 percent; 2) Tween 20* at 0.10 percent; or 3) Romx-C* at 0.10 percent. The concentration of gibberellin within the solution is given as the weight of GA to the final, total volume of the solution and is specified as mg l^{-1} GA (e.g., 600 mg l^{-1} GA).

Foliar spray application may be done with spray bottles. Typically, all foliage of the crown or branch to be treated is thoroughly wetted with the GA solution until nearly or actually dripping. The comparison of works dealing with foliar applications must be based on the assumptions that all surfactants are equally effective, and that there are no species differences in response to any one surfactant (which may not be the case).

It is worth noting that the Aromox-ethanol solution as a control (i.e., with no GA) versus an untreated control, does not affect flowering (Wheeler et al. 1980, Ross et al. 1981, Cecich 1983). This is useful from the point of experimental design, for it suggests that there is no need to have an untreated control.

Topical application - For the purpose of topical application, gibberellin is dissolved in a solvent solution of ethanol and water which may vary from 50:50 ethanol:water to 100:0; a typical ratio is 80:20 ethanol:water. The concentration of GA is usually specified as μg GA per branch or bud (e.g., $500 \mu\text{g bud}^{-1}$). Only small amounts of the GA solution need to be applied to achieve the desired dosage; for example, $10 \mu\text{g bud}^{-1}$ is a common rate. Application is done with an appropriate pipette or a micro-syringe. Topical application of GA is more labor intensive than foliar application, but it does provide a more direct estimate of the amount of GA being applied to the tree.

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Stem or branch injection - The GA solution used for stem or branch injection is similar to that used for foliar spraying. A specified weight of GA is dissolved in an ethanol:water solution, but no surfactant is added. The concentration is typically specified in mg l^{-1} GA. It is necessary to specify either the amount of solution which was taken up by the tree or, more directly, the actual amount of GA (i.e., mg GA tree^{-1}). Injection of gibberellin directly into the tree may be accomplished by one of two means:

- 1) Cut branch injection. A lateral branch is cleanly severed and tubing is slipped over the cut end. The other end of the tube is attached to an intravenous bottle containing the GA solution. The bottle is then inverted and positioned above the branch to allow uptake of the solution. The branch may have to be re-cut as wound reaction inhibits uptake (e.g., Puritch and McMullan 1981, Greenwood 1982).
- 2) Stem injection. Holes are drilled slanting downwards into the stem. The GA solution is injected into the holes with a hypodermic syringe or applied using an intravenous delivery system. This technique may be ineffective with species of *Pinus* owing to resin buildup at the injection hole (Wheeler et al. 1980).

Comparison of application techniques - A few studies have compared the effectiveness of the various application techniques. For example, Greenwood (1982), working with 3-year-old potted grafts of loblolly pine, observed that neither foliar spray application nor the cut branch technique was successful, whereas topical application was successful. Working with 12-year-old field grafts of the same species, Hare (1984) found that foliar spray applications of GA were more effective than topical bud treatments in enhancing flowering.

Ross et al. (1981) showed that foliar sprays were as effective as stem injections for promoting flowering in western hemlock, while being less phytotoxic as well. Stem injections gave only slightly better results than

foliar applications in Japanese larch and European larch (Bonnet-Masimbert 1982). In Douglas fir, however, stem injections were consistently more effective than foliar sprays (Ross and Pharis 1982).

Concentration of Gibberellin

Studies have shown that there are sub-optimal, optimal, and supra-optimal concentrations of GA. Tompsett and Fletcher (1979) observed an optimum concentration of GA for both male and female flowering of 11-year-grafted mature scions of Sitka spruce. The best male flowering response occurred at $19.7 \text{ mg ml}^{-1} \text{ branch}^{-1}$; at concentrations above and below that level, flowering response diminished. A supra-optimal concentration was also noted in the female flowering response of 9-year-old white spruce seedlings (Marquard and Hanover 1984a). Foliar applications of 500 mg l^{-1} produced fewer female strobili than 250 mg l^{-1} . A very similar response was noted by Marquard and Hanover (1985) when GA was applied to 6-year-old white spruce seedlings with certain adjunct cultural treatments.

Cecich (1983) sprayed 3-year-old accelerated growth seedlings of jack pine with 200, 400, and 600 mg l^{-1} and observed that both male and female flowering response was greatest at the highest concentration. Similarly, Ho (1985), working with 15-year-old grafts of white spruce, found that $\text{GA}_{4/7}$ at a concentration of 800 mg l^{-1} produced significantly more female strobili than either 200 or 400 mg l^{-1} GA.

Ross et al. (1981) showed that the flowering response of 7- and 8-year-old rooted ramets of mature western hemlock was roughly proportional to the logarithm of the $\text{GA}_{4/7}$ concentration. Similar results were noted by Greenwood (1982) for 3-year-grafted mature scions of loblolly pine. Though the highest dosage, $500 \mu\text{g GA}_{4/7} \text{ bud}^{-1}$, provided the greatest flowering response, increasing the dosage by 150 percent (from 200 to $500 \mu\text{g bud}^{-1}$) increased flowering by only 13 percent. A logarithmic dose-response curve was also observed for Douglas-fir (Ross 1983).

Timing of Application

One of the most important factors to consider in the application of gibberellins (or adjunct cultural treatments) is the timing of the application. The optimum time and duration of application will vary with tree species, genotype, and age, as well as cultural and environmental factors that affect tree growth and phenology. This is largely due to the effect these factors have on conebud development.

In *Pinus*, *Abies* and *Pseudotsuga*, cone buds develop from newly initiated axillary apices. Cone buds of *Picea* and *Tsuga* similarly develop from axillary apices, but also from terminal, previously vegetative, apices. In most of these genera (*Abies*, *Picea*, *Tsuga*, and *Pseudotsuga*), the axillary apices are initiated in the axils of leaves on elongating shoots (Owens and Blake 1985). In *Pinus*, axillary buds are initiated in the axils of cataphylls within a complex long shoot bud (e.g., Greenwood 1980). Cone buds of *Larix* normally develop from terminal apices of vegetative short shoots (Owens and Molder 1979).

The development of terminal and axillary apices begins with the initiation of a series of bud-scales. Anatomical differentiation of an apex is marked by the shift from bud-scale initiation to the initiation of foliar organs: needles, bracts, or microsporophylls. This period is preceded by biochemical differentiation (Owens and Blake 1985). It is during this time that the bud type (vegetative, seed-cone, or pollen-cone) is determined.

In several species, the period of bud-type differentiation is coincident with a specific phase of shoot elongation. For example, anatomical differentiation of cone buds in *Pseudotsuga* and *Tsuga* occurs shortly after vegetative shoots have flushed, whereas in *Picea* and *Larix*, it coincides with the completion (the latter, slow phase) of shoot elongation (see references Harrison and Owens 1983, Owens and Blake 1985). In *Pinus*, cone bud differentiation occurs after shoot elongation is complete (Greenwood 1980). It is therefore possible (and advisable) to use shoot elongation as the timing criteria for gibberellin application, rather than calendar dates alone, since it more accurately reflects genetically and environmentally related seasonal variation in plant development (Ross 1985).

A number of studies have revealed the significance of the timing of application (though most of these cite calendar dates to indicate timing). For example, Tompsett and Fletcher (1979) applied gibberellins ($0.6 \text{ mg GA}_{4/7} + 0.6 \text{ mg GA}_9 \text{ branch}^{-1}$) at weekly intervals to 2-year-grafted mature scions of Sitka spruce in a polyethylene house. This was done over all combinations of three treatment periods: May 20 to June 24, July 1 to August 5, and August 12 to September 16. The level of male flowering increased with the number of treatment periods, response being greatest when application was made over all three periods, least when made over single periods only. Furthermore, the four treatment combinations that included the first treatment period were generally much more effective than those that did not.

Marquard and Hanover (1984a) sprayed white spruce (250 and $500 \text{ mg l}^{-1} \text{ GA}_{4/7}$) in three weekly applications, initiating treatments at four different times. Female flowering was maximized by treatments initiated on June 2. Treatments initiated on May 13 were somewhat less successful; those initiated on June 23 and July 15 were ineffective. Shoot growth cessation and meristematic differentiation occurred in the second half of June. Ho (1985), also working with white spruce, found that biweekly spray application of $\text{GA}_{4/7}$ ($200 \text{ mg l}^{-1} \text{ GA}_{4/7}$) from May to July significantly enhanced female strobili production, whereas application from June to August did not.

Cecich (1985) showed that weekly spray applications of $600 \text{ mg l}^{-1} \text{ GA}_{4/7}$ from May 7 to July 1 significantly increased the number of female and male strobili of 12-year-old white spruce; applications from July 8 to September 9 produced no strobili. Subsequently, he found that applications from May 13 to June 3 produced more strobili than application from June 10 to July 1, even though 95 percent of total shoot elongation was reached at the end of June/beginning of July. He suggested that "Perhaps foliar spray of $\text{GA}_{4/7}$ was more effective during May because the assumed penetration and uptake of the spray solution was easier in young succulent needles than in

older ones during the June treatment. Alternatively, a possible lag effect to GA_{4/7} that is applied by spray may account for the greater flowering response of the May treatment versus the treatment in June".

Working with European and Japanese larches, Bonnet-Masimbert (1982) showed that the optimum treatment period for spray and stem injection of GA_{4/7} was around May and June.

The female flowering of loblolly pine was affected about equally by biweekly GA_{4/7} (500 μ g bud⁻¹ GA_{4/7}) applications performed from June 18 to August 13 and August 13 to October 22 (Greenwood 1982). Application over both of these periods, however, provided the best results. Initiation of female primordia of loblolly pine occurs in the latter half of August (Greenwood 1980).

Effects of timing of treatment on male:female strobilus ratio - Timing of the gibberellin treatment may affect the proportion of male and female strobili that are produced in species in which the two differentiate at different times. For example, Chalupka (1978), working with 15-year-grafted scions of Scots pine (*Pinus sylvestris* L.), observed that three weekly applications of GA_{4/7} in May significantly increased male, but not female flowering. Chalupka (1980) concluded that, for pines in general, the early application of GA_{4/7} (in the first part of the vegetative season, May and June) promotes male flowering. Extending the application into July, August and September promotes female flowering. He suggested that this reflects the earlier differentiation of male strobili, and further, that this may be related to the fact that initiation and development of cone buds occurs proximally on the developing shoot for males and distally for females.

Work with western hemlock (Ross et al. 1981) and Engelmann spruce (Brix and Portlock 1982) similarly suggests that for these species, earlier treatment application may preferentially promote male strobili production.

Interaction between timing of treatment and crown zonation - The optimum time for GA application and treatment effects on sexuality may also vary with crown level. The crown of a mature conifer may be spatially divided into three zones: 1) the upper crown, which bears predominantly female flowers; 2) the lower crown, which bears predominantly male flowers; and 3) the transitional zone located between these two, which may bear both male and female flowers. These zones may respond differently to gibberellin application.

Chalupka (1980) showed that spraying $GA_{4/7}$ from May to August significantly increased the production of female strobili (but not male strobili) of Scots pine, the response being most apparent in the middle and lower crown regions. Conversely, enhanced male (but not female) flowering of Scots pine, in response to $GA_{4/7}$ applied in May and June, was realized only in the upper and lower crown regions, not in the middle region (Chalupka 1981).

Bonnet-Masimbert (1982) was also able to stimulate female flowering in the lower, male zone of European and Japanese larch using $GA_{4/7}$. He also showed that the optimum treatment period for enhancing female flowering of upper branches was approximately one month later than that of lower branches.

Hare et al. (1977) observed that application of $GA_{4/7}$ + naphthalene acetic acid (NAA) enhanced the female flowering response in both low and mid to upper crown regions of longleaf pine. Male flowering was only slightly enhanced in the upper crown, and not at all in the lower crown. The female strobilus production of white spruce was also shifted to lower crown regions by $GA_{4/7}$ applications (Ho 1985).

Working with white spruce, Marquard and Hanover (1984b) observed that male and female flowering in the transitional zone significantly increased in response to $GA_{4/7}$; in the male zone, the number of male strobili increased, while female strobili were induced as well. Neither male nor female flower production, however, were enhanced in the upper female zone

of the crown by $GA_{4/7}$. These workers suggest that hormonal or nutritional gradients might exist within the crown such that endogenous factors necessary for flower production in the upper crown are near optimal and not readily enhanced by exogenous application of $GA_{4/7}$. Marquard and Hanover (1984a) also showed that $GA_{4/7}$ application shifted the within-shoot distribution of female strobili to more distal shoot positions, suggesting the presence of endogenous hormonal or chemical gradients within shoots as well. The shift of female strobilus production to lower portions of the crown or to more distal shoot positions in response to $GA_{4/7}$ may reflect changes in these gradients (Ho 1985).

It is worth noting that the timing of treatments may affect the ratio of male to female strobili, even if both are differentiated at the same stage of shoot development. As previously suggested, male strobili are typically found on the less vigorous shoots of the lower crown, whereas female strobili are associated with the more vigorous shoots of the upper crown. Lower branches may thus complete development before branches in the upper crown. Consequently, if differentiation occurs at the same stage of shoot development, female buds may differentiate after and over a longer period of time than male buds (Ross et al. 1981, Bonnet-Masimbert 1982, Ross 1985).

Adjunct Cultural Treatments

There are several cultural treatments that have been used in attempts to promote flowering in conifers. These include water stress, root pruning, heat treatment, girdling, out-of-phase dormancy, and fertilization. Often these treatments are applied as secondary factors to gibberellin application; they may in fact prove ineffective alone. Conversely, in some cases the application of GA alone fails to promote flowering, though a response is realized when it is applied with an adjunct treatment. Thus, it is important to consider the potential of each treatment alone and how it interacts with other inductive treatments, particularly GA, to affect flowering.

Water Stress

Water stress is induced by limiting soil moisture, usually through a series of drying cycles. A drying cycle involves watering a tree to field capacity, allowing the soil to dry out until a specified critical water potential within the tree is reached, and then re-watering to field capacity again. Two variables used in the application of a water stress treatment are the intensity and duration of the water stress. Intensity is determined by the critical water potential used to mark the point at which re-watering will be done. This factor, in combination with the rate of transpiration, will determine the length of the drying cycles. The duration of the water stress is simply a factor of the number of drying cycles which are applied, i.e., the length of the treatment period.

For several reasons, the length of the drying cycles may vary from tree to tree and from one cycle to the next. For example, plants in a greenhouse will typically transpire more than comparable plants outside, and may therefore reach the critical water potential sooner (e.g., Greenwood 1981).

If the same watering regime were used for both the indoor and outdoor plants in this case, the former would actually be under greater water stress. Similarly, a large potted tree will more quickly utilize available soil moisture than a small tree in the same-sized pot. The drying cycle of the larger tree will be shorter, necessitating more frequent watering if stress levels of the two trees are to be comparable. Clonal variation in water-use efficiency may also require consideration.

The effective application of water stress requires the ability to monitor the water status within the tree. This is typically measured using a pressure bomb and is recorded as the xylem or needle water potential. A technique commonly used is the measure of the force required to extract a drop of moisture from a freshly cut needle or needle fascicle cross-section. This provides an estimate of the internal moisture stress of the tree. The measure is taken in megapascals (MPa). Examples of critical water potential values at which re-watering may be applied range from -0.51 to -1.70 MPa (Greenwood 1981, Ross et al. 1981, Ross 1985). Water potential values are usually measured at predawn hours (e.g., 6:00 a.m.).

There are several examples of the effectiveness of water stress as an induction treatment. Ross et al. (1981), working with potted 3-year-old seedlings of western hemlock, applied water stress from June 17 to August 16, with concurrent weekly $GA_{4/7}$ (200 mg l⁻¹) spraying beginning July 8. While $GA_{4/7}$ alone did not promote flowering, $GA_{4/7}$ applied with water stress significantly enhanced female flowering and to a limited extent, male flowering as well. In this regard, the authors note that water stress was more effective than girdling.

In a similar study, Brix and Portlock (1982) applied one to three drying cycles to 1- to 4-year-old seedlings of western hemlock, during the period of rapid shoot elongation (June 15 to July 31). Water stress of any duration alone promoted neither male nor female flowering; weekly sprays of $GA_{4/7}$ (200 mg l⁻¹) also proved ineffective. The two treatments combined, however, significantly enhanced both male and female strobilus production.

To a certain degree flowering response increased with the duration of the stress cycle. Brix and Portlock (1982) point out that the successful induction of male strobili they observed, relative to the results of Ross et al. (1981), may reflect differences in the timing of $GA_{4/7}$ application. They suggest that July 8 may have been too late to affect differentiation of male strobili, which in western hemlock occurs in late June; differentiation of female strobili occurs in early July (Owens and Molder 1974).

Water stress applied from mid-July to September with biweekly $GA_{4/7}$ (200 or 500 μg bud⁻¹) applications increased female flowering of 2- to 6-year-old grafted scions of loblolly pine, though male flowering was not enhanced (Greenwood 1981). The timing of the $GA_{4/7}$ treatment bracketed the period of male strobilus differentiation (Greenwood 1980).

Working with 5-year-grafted mature scions of Engelmann spruce, Ross (1985) applied water stress for all combinations of three stages of shoot elongation: early-slow, rapid, late-slow. The female flowering response was generally greatest for drought treatments which included the period of rapid shoot elongation (a period which precedes apical differentiation). Drought applied only in the late stage of shoot elongation inhibited flower production. The male flowering response to drought was wanting, though enhanced to some extent with the application of $GA_{4/7}$. Olsen (1978, cited by Ross 1985) also noted that drought applied during the late stage of shoot elongation inhibited flowering of Norway spruce.

Root Pruning

Another cultural treatment which has proven to be effective for flower induction is root pruning. This technique typically involves severing the root system at a specific depth or distance from the tree. Various devices have been used to carry out root pruning, including hand spades, tree spades, and transplanters (e.g., Masters 1981, Marquard and Hanover 1985, Ross et al. 1985b).

An example of this technique may be found in the work of Marquard and Hanover (1985), who root pruned 6-year-old white spruce seedlings. Though it had no effect on male strobilus production, root pruning significantly increased both female strobilus production per tree and the number of trees producing female strobili. A $GA_{4/7}$ by root pruning interaction was not significant, suggesting the lack of synergism between these two treatments.

A similar study was carried out by Ross et al. (1985b) on both mature grafts and 9-year-old seedlings of Douglas-fir. A significant increase in female flowering in response to root pruning was realized on both seedlings and grafts, whereas male flowering was enhanced on the grafts only. In this case, however, a synergistic effect was noted when $GA_{4/7}$ was applied in conjunction with root pruning. When applied separately, root pruning was more effective than $GA_{4/7}$ for promoting female flowering of 9- and 10-year-old Douglas-fir (Webber et al. 1985). In combination, these two treatments produced a highly significant synergistic response. Several other studies have also shown that root pruning and $GA_{4/7}$ can have a strong synergistic effect when combined (*see* references Ross et al. 1985b).

Root pruning is typically applied no more than once in a growing season. Concern has been expressed that repeated, annual treatment may prove deleterious, in terms of both general tree health and seed yield (e.g., Sweet 1975). More effective and consistent seed production may be achieved where root pruning is applied biennially, and where trees are well watered and fertilized in the year following treatment (*see* Ross and Pharis 1982).

In regard to the timing of root pruning, good results have been obtained where treatment preceded vegetative bud break by four to six weeks, i.e., approximately late March, early April (e.g., Marquard and Hanover 1985, Ross et al. 1985b). Root pruning at the time of vegetative bud swelling (mid-April) significantly increased flowering of grafts and seedlings of Douglas-fir (Masters et al. 1981, Webber et al. 1985). In addition, Masters et al. (1981) showed that pruning at the end of the period of rapid shoot elongation (June 26) was largely ineffective. Similarly, Heitmüller and

Melchior (1960, cited by Matthews 1963) observed that root pruning applied in June enhanced neither the female nor male flowering response of 4-year-old grafts of Japanese larch.

Heat Treatment

The effect of heat treatment on flower induction has been studied in several conifer species. Heat treatments may be applied by covering individual trees (or parts thereof) with polyethylene covers (e.g., Chalupka and Giertych 1977; Chalupka 1981), or by moving potted trees into a greenhouse (e.g., Tompsett and Fletcher 1977, 1979; Ross 1985).

Chalupka and Giertych (1977) increased the number of male flowers per graft by covering Norway spruce of two sources with polyethylene sleeves from June 3 to July 17. The optimum period of treatment for the northern source was June 3 to July 5, and for the southern source, June 3 to July 17. Neither female flowers per graft nor number of grafts bearing male or female flowers were increased by this treatment. Chalupka and Giertych (1977) suggest that the difference in male and female flowering reflects the earlier differentiation of the male strobili; the clone by treatment interaction may reflect the later differentiation of the male strobili of clones from the southern source. Chalupka (1981) also observed enhanced male, but not female, flowering by covering grafts of Norway spruce with polyethylene from June 18 to July 24.

Tompsett and Fletcher (1977) observed significant increases in the male and female flowering of 4-year-grafted mature scions of Sitka spruce placed in a polyethylene house, a response they attributed to higher temperatures. Similarly, by placing 5-year-grafted material in a polyethylene house, Tompsett and Fletcher (1979) found that high temperatures increased the male flowering response of the same species. In this case, a June and July treatment was more effective than treatment in August and September; the best response, however, was achieved by keeping grafts indoors over both periods.

More recently, Pollard and Portlock (1984), working with 3-year-old seedlings of western hemlock compared the effects of two constant temperatures (20°C and 30°C) applied from April 22 to June 17, as an adjunct treatment to weekly applications of $200 \text{ mg l}^{-1} \text{ GA}_{4/7}$. Both male and female flowering were greater at the higher temperatures, though the difference was significant only for the latter.

Ross (1985) observed that high temperatures ($30^{\circ}\text{C}:20^{\circ}\text{C}$ day:night) applied to Englemann spruce during the late-slow stage of shoot elongation enhanced female flower production. Male flowering was enhanced to a great extent by high temperatures applied during both the rapid and late-slow stages. Both the male and female response to heat stress was improved by concurrent applications of $\text{GA}_{4/7}$.

Girdling

Girdling has frequently been used to enhance flowering, both alone and as an adjunct cultural treatment to gibberellin application. For the purpose of flower induction, girdles do not usually completely encircle the stem. Rather, they typically consist of two disconnected half-circumferential cuts, which sever the cambium and phloem, on opposite sides of the stem or branch. These two cuts are spaced apart along the stem or branch, but the ends overlap to varying degrees. The variables involved in girdling studies, due to the girdling itself, are: 1) the width of the cut (W); 2) the amount of overlap of the cuts on either side of the stem (O); 3) the distance between the two cuts (D); and 4) the height of the cut above the ground (H) (letters in brackets will be used to subsequently refer to these variables). Rather than absolute units, the measure D is sometimes derived as a proportion of the stem or branch diameter and may thus vary with tree size (e.g., Wheeler et al. 1985, who specified that $D=1.5$ to $2.0 \times$ stem diameter).

Ebell (1971) determined that the optimum time for girdling of Douglas-fir was one month prior to vegetative bud break and coincident with the expansion of pollen buds (a period which precedes bud differentiation by several weeks). Wheeler et al. (1985) also achieved good flowering response by girdling at this time. These workers also noted that: 1) over a four-year period, annual girdling resulted in greater seed cone production than biennial girdles, and 2) saw-cut girdles ($W=3-5$ mm) were generally more efficient than band girdles ($W=25$ mm). Ho (1985) significantly increased female strobilus production of 15-year-old Norway spruce using stem girdles in early May ($W=2$ mm, $D=30$ cm, $O=3$ cm). Several workers have suggested that girdling should be done at the time of bud initiation (see references Sweet 1975).

Hare et al. (1977) observed that branch girdles ($D=1$ cm, $W=1$ cm) together with ($GA_{4/7} + NAA$) application enhanced female strobilus production better than either treatment alone, in both the upper and lower crown of 13-year-old longleaf pine. Girdling alone proved the most efficacious in the upper crown, while in the lower crown, girdling, ($GA_{4/7} + NAA$), and complete branch girdles ($W=5$ mm), Chalupka (1978) was able to significantly enhance male and female flowering of 15-year-old Scots pine grafts. Though a girdle by $GA_{4/7}$ treatment interaction was not significant, a synergistic effect was evident in the male flowering response to these two treatments. Bonnet-Masimbert (1982) applied girdles ($W=8$ mm, $D=50$ cm, $H=40$ cm) to European and Japanese larches two to five days prior to the initiation of $GA_{4/7}$ treatments. Girdling enhanced both the male and female flowering response to $GA_{4/7}$. Wheeler et al. (1980) found that stem and branch girdles ($W=0.5$ cm, $D=2$ cm, $H=0.5$ m), in conjunction with $GA_{4/7}$ application, increased male and female strobilus production of 6- to 11-year-old seedlings of lodgepole pine.

Pharis et al. (1980) girdled branches ($W=5$ mm, $D=10$ mm) of 6-year-old seedlings of Douglas-fir in early April, one week before concurrent hormone treatments were initiated. Girdling alone had a small but significant effect on male and female strobili production. Philipson (1985) girdled ($W=13$ mm, $D=30$ mm, $O=10-12$ mm) 14-year-old sitka spruce grafts on May 20, at which

time vegetative shoots were flushing (reaching full extension by July 14). Alone, girdling did not significantly increase the male and female flowering. It did, however, significantly increase the flowering response to $GA_{4/7}$. Evidence also indicated a carry-over effect of girdling and $GA_{4/7}$ into the second year after treatment. Philipson (1985) suggested that though it is often ineffective alone, girdling can increase the flowering response elicited by $GA_{4/7}$.

It should be noted that girdling may or may not have deleterious effects on tree growth and seed yield. Girdling reduced yields of filled seed of Douglas-fir (Ross et al. 1980). Wheeler et al. (1985), however, noted no decline in seed quality of Douglas-fir even after four years of annual girdling. Philipson (1985) observed that 11 of 30 trees treated with complete girdles broke at the girdle by the second year after treatment. Conversely, Wheeler et al. (1985) observed that only 1 of 421 Douglas-fir trees given disconnected girdles broke at the girdle. Girdling may reduce height growth (Wheeler et al. 1985), cause chlorosis of foliage (Ho 1985, Philipson 1985) or, in the case of branch girdles, result in the death of branches (Hare 1978, Hare et al. 1977). Some of these effects may be temporary. For example, girdling of the main stem of lodgepole pine caused slight stunting of initial shoot and needle growth the following season, but recovery occurred by mid-summer (Wheeler et al. 1980.)

Out-of-Phase Dormancy

An interesting flower induction technique which has not yet been widely applied is out-of-phase dormancy. The technique as it may be applied to loblolly pine is described by Greenwood (1978, 1981) and O'Gywnn (1983b). Treatment begins in late summer: actively growing trees are provided an extended photoperiod in a heated greenhouse to maintain active shoot growth and inhibit dormancy. In mid-winter/early spring, the supplemental lighting is discontinued and trees are subjected to the photoperiod provided by natural daylength. At this time, the temperatures may be reduced or

maintained at previous levels. In either case, the effect of the reduced photoperiod is to reduce shoot growth and induce the formation of a quiescent or resting bud, which will develop male and female strobili (Greenwood 1981).

Applying out-of-phase dormancy to loblolly pine, Greenwood (1978) promoted male and female strobilus production in grafts of 3- to 7-year-old scions; 38 and 68 percent of ramets produced female and male strobili, respectively. Similar responses were observed by Greenwood (1981) using grafts of 4- to 12-year-old scions. In this case as well, out-of-phase dormancy proved most effective in enhancing male flowering.

Fertilization

Fertilization has proven to be another means by which flowering of some species may be enhanced. Since fertilizers are relatively easy to apply on a large scale, they have frequently been used in seed orchards and in natural stands. The same principles and practices may, however, find application in indoor breeding culture as well. Specifically, fertilization may be applied to enhance the effects of other induction treatments.

Gregory et al. (1982) studied the effects of N, P, and K fertilization and irrigation on female flowering in a loblolly pine clonal seed orchard. They found that both fertilization and irrigation alone markedly enhanced flowering, but the greatest and only significant response was elicited by a combined application of both. Also working with loblolly pine, Schmidtling (1975) showed that N fertilization alone was as effective as N + P in enhancing female flowering. Conversely, Steinbrenner et al. (1960, cited by Sprague et al. 1978) found that N + P was more effective than N alone for Douglas-fir.

Heidmann et al. (1978), working with a 55-year-old natural stand of ponderosa pine (*Pinus ponderosa* Laws.), observed a significant positive linear relationship between level of N + P fertilization applied in 1974 and

cone production in 1976. In addition conelet abortion decreased with increasing fertilizer application. The authors point out that the fertilizer was applied after bud differentiation and suggest that the enhanced cone production was largely a reflection of the reduction in conelet abortion. "... Abortion is a constant process that starts soon after differentiation of flower initials and continues after pollination. Abortion may be directly affected by the physical condition of the shoot and subject indirectly to cultural treatments that alter this condition" (Heidmann et al. 1978). In contrast, however, Summerville et al. (1978) found that fertilization and irrigation (alone or in combination) did not significantly reduce conelet abortion in a 7-year-old longleaf pine clonal seed orchard.

In regard to time of application, Schmidtling (1975) found that flowering was maximized by fertilization applied from mid- to late summer. He recommended that "fertilizers should be applied early enough to increase flower initiation (or perhaps prevent abortion of already formed primordia) but late enough to prevent uptake by competitors or diversion of nutrients to growth rather than flowering." A review of management practices for southern pine seed orchards also lead Sprague et al. (1978) to conclude that the optimum time for fertilizer application is in late July or early August.

NATURAL SOURCES OF VARIATION IN PRECOCITY AND FRUITFULNESS

Comparisons of various flower induction studies are difficult because both precocity and fruitfulness may be subject to several sources of variation, independent of treatment effects. These sources may include:

- 1) type of stock used: seedling or graft,
- 2) species,
- 3) genotype, and
- 4) age and vigour.

In the case of grafts, it is also necessary to consider points 2 to 4 above in terms of both scion and rootstock, as well as any interaction between the two.

Genetic Variation

In attempting to induce precocious or enhanced flowering, it is important to realize that genetic variation likely constitutes a significant proportion of the total observed variation, regardless of the treatment being applied. Recognition of this source of variation is important to accelerated breeding programs for at least three reasons: 1) it might be possible to select individuals that are predisposed to precocious or enhanced fruitfulness; 2) incorporating clone or genotype as a factor in experimental designs will make for more efficient experiments in flower induction that are more sensitive to real treatment effects; and 3) by considering genotype, it will also be possible to estimate genotype by treatment interactions.

In regard to this last point, a significant clone by treatment interaction would suggest that the magnitude or direction of the treatment response differs among clones. It would therefore be necessary to consider the effects of a treatment as it applied to each individual clone. Conversely, the lack of a significant interaction would indicate that treatment response is essentially independent of the genotype to which it is applied (in regard to those genotypes tested).

Evidence suggests that fruitfulness and precocious flowering are under moderate to strong genetic control. Estimates of broad-sense heritability for fruitfulness were 0.5 for slash pine and ranged from 0.4 to 0.7 for loblolly pine (*see* references Schmidtling 1974). Schmidtling's (1981) estimates of the narrow-sense heritability for flowering in loblolly pine were 0.61 and 0.52.

Schmidtling (1981) and Heimbürger and Fowler (1969) concluded that precocious flowering was a heritable trait for which selection could be effective. Schmidtling (1981), however, suggested that this was inadvisable owing to a negative correlation between flowering traits and growth traits. Similar concerns were addressed by Bramlett and Belanger (1976) who cite some studies which indicate that generative and vegetative growth may be negatively correlated, and others which show zero or positive correlations between these traits. They suggest that while selection for vegetative growth should be emphasized, a minimum flowering potential should be maintained.

Sweet (1975) stressed the importance of considering clonal variation in flower production when selecting individuals for breeding orchards. His review led him to conclude that induction treatments are most effective on individuals genetically predisposed to precocious or enhanced flowering. More recent work has shown that this may be the case with several conifers, including loblolly pine (Schmidtling 1975), Norway spruce (Dunberg 1980), lodgepole pine (Wheeler et al. 1980), western hemlock (Ross et al. 1981), and Douglas-fir (Ross et al. 1985b).

Many studies clearly show the significance of genotype in determining flowering response. For example, Chalupka (1978) observed significant clonal variation in the male flowering of Scots pine. Clones also exhibited a differential flowering (both male and female) response to induction treatments which included $GA_{4/7}$ and girdling; several treatment by clone interactions were significant. Chalupka (1981) also noted significant clonal variation, though no treatment by clone interaction, in the male flowering of Norway spruce. Scots pine, on the other hand, exhibited significant clonal variation as well as significant treatment ($GA_{4/7}$, crown position) by clone interactions, though in regard to male flowering only.

In another study with Scots pine, Luukkanen and Johansson (1980) observed a clone by year interaction; some individual clones exhibited similar responses in each of two consecutive years, others reacted differently over the same two years. They also found that "... an initial low level of female flowering seems to depend either on a permanent tendency to poor female flower development which is not affected by exogenous gibberellins, or on environmentally determined fluctuations in female flowering which, during a year of poor flowering, can be increased using a GA application "... male flowering on untreated grafts or after GA treatment did not show any clear relation to the earlier classification of clones according to their female flowering or cone producing tendency."

Ho (1985), working with white and Norway spruce, found that 26 to 34 percent of the variation in female strobili production was associated with clones. Treatment ($GA_{4/7}$ and girdling) by clone interactions were not significant, accounting for 10 to 17 percent of total variation. Dunberg (1980) observed "great differences" in the flowering response of 15-year-old grafts of Norway spruce (to various gibberellins) among clones and even among grafts of the same clone.

Wheeler et al. (1980) found significant clonal variation, but no treatment ($GA_{4/7}$) by clone interaction in the female flowering response of lodgepole pine. Schmidting (1975) and Gregory et al. (1982), working with loblolly pine, and Summerville et al. (1978), working with longleaf

pine, observed significant clone and clone by treatment interactions in flowering response to fertilization and irrigation. In addition to yearly fluctuations, a significant treatment by year interaction was noted (Gregory et al. 1982). This suggests that the magnitude of the differences in treatment response varied with year of application. In addition, Summerville et al. (1978) showed that there were large differences in flower production among ramets within a clone and that the variability among ramets of a clone varied markedly with clone (i.e., the magnitude of variation within clones varied among clones).

Age of Material

The age of seedling or scion material may profoundly affect its potential flowering level. For example, Bleymuller (1976) found that spray application of GA_3 + CCC ((2-chloroethyl)-trimethyl-ammonium chloride) stimulated female flowering in 16-year-old Norway spruce trees but not in 3-year-old seedlings or grafts. Working with loblolly pine, Ross and Greenwood (1979) also observed that the female flowering response of 45-year-old grafted scions was much greater than that of 8- to 10-year-old scions. A similar response in the male flowering of this species has also been noted (Greenwood, unpublished as cited by Ross and Greenwood 1979).

Greenwood (1981) grafted 1- to 12-year-old scions of loblolly pine onto uniform root stocks and found that strobilus production increased significantly with scion age. He also observed that the number of growth cycles and shoot elongation decreased with age. Ross et al. (1981) observed that of 1- to 4-year-old seedlings of western hemlock, the younger seedlings were less responsive to gibberellin applications. Similarly, Ross (1983) showed that the flowering of grafts and rooted cuttings of Douglas-fir in response to $GA_{4/7}$ increased with the increasing age of propagules.

These observations support Marquard and Hanover's (1984a) suggestion that increased age may reduce the level of exogenous $GA_{4/7}$ required to elicit female flowering. This may also be the case for male flowering, as suggested in the work of Greenwood (1981). A possible physiological basis for this observation is a natural increase in less polar endogenous gibberellins associated with increasing tree age (see Ross 1983).

Scion - Rootstock Interaction

The combination of scion and rootstock may also have a profound effect on the growth and flowering of grafts. Points to consider include age, vigour, species and genotype of both scion and stock. For example, in his study of intra- and inter-specific scion-rootstock combinations, Ahlgren (1962) grafted scions of several pine species onto rootstock of both white pine and red pine. Female cone production was greatest on the rapidly growing rootstocks. For some scion-rootstock combinations, flowering increased markedly with the age of the rootstock.

In a similar study, Schmidting (1983) examined the growth and flowering of loblolly pine scions grafted onto stocks of several different pine species. He observed that flowering, growth and survival of the grafts was significantly affected by rootstocks, and concluded that selection of rootstock on the basis of precocity may be used to promote early and enhanced flowering of grafts. Apparently, the natural flowering tendency of the rootstock species and genotype may influence the flowering of the graft. Simak (1978), working with Scots pine, also showed that flower induction in grafts may be affected by scion-rootstock combinations as well as graft type.

SUMMARY

It is clear that indoor breeding culture can be effectively applied for two distinct purposes: to accelerate early seedling growth, and to promote precocious and enhanced flowering. Accelerated seedling growth is achieved through an intensive cultural program which is based on an extended photoperiod in conjunction with, among other things, optimal temperature, nutrient and moisture conditions. The potential of this type of program is evidenced in its ability to produce plantable seedlings in two to three months rather than two to three years. In some cases, precocious flowering may also be realized. These factors can mean more rapid and efficient genetic testing and a reduction in the time required to produce and test desired crosses. For production purposes, the advantages afforded by accelerated growth are also substantial: the production of larger seedlings of better quality, and with better growth and survival after outplanting.

While precocious and enhanced flowering of seedlings may be a consequence of accelerated growth, these aims may also be achieved independently, and perhaps more effectively, through the judicious application of hormonal and cultural treatments. This is particularly true of grafted mature stock which may not respond to accelerated growth treatments. One of the problems, however, in coming to general conclusions about flower induction techniques is that marked variability exists in the flowering response that these treatments elicit. Nevertheless, some results do seem more or less consistent and warrant consideration.

The most effective induction treatments seem to be those that include gibberellin. The mixture of GA_{4/7} seems particularly efficacious. The literature suggests that little is likely to be gained in the testing or use

of the other gibberellins (i.e., GA₃, GA₅, and GA₉) with members of *Pinaceae*. Even GA_{4/7}, however, may prove ineffective alone. Best results will likely be obtained with GA_{4/7} application in conjunction with adjunct cultural treatments. Both heat treatment and water stress are relatively easily applied under greenhouse conditions, and both have proven effective when applied with GA_{4/7}. Root pruning or girdling in combination with GA_{4/7} may also be effective, though deleterious effects due to girdling have been noted. Out-of-phase dormancy is an interesting treatment which has proven useful in promoting precocious flowering in loblolly pine; it warrants testing with other species.

Regardless of the treatment to be used, the timing of application can make the difference between success and failure. Most successful induction treatments seem to exert their effect by influencing the differentiation of already initiated bud primordia (fertilization may be an exception). Thus, treatments must be applied sometime before bud differentiation occurs. Among several species of *Pinaceae*, the time of bud differentiation is approximately coincident with a certain phase of shoot elongation. Shoot elongation has successfully been used as a criteria to estimate the optimum time for GA_{4/7} application in these species. The optimal timing of cultural treatments is not as clear, owing likely to the complex pathways through which their effect is realized. Successful treatment applications cited herein may provide some guidelines.

It is clear that genetic variation has a significant effect on precocity and fruitfulness. Any experiment designed to test treatment effects on flowering must incorporate genotype and genotype by treatment interaction effects. Selection could be used to enhance flowering traits, but in at least some species, this would be at the expense of vegetative growth. Since most species will respond to some form of flower induction treatment, it seems best to emphasize selection for vegetative traits instead. A minimum flowering potential should, however, be maintained.

LITERATURE CITED

- Ahlgren, C. E. 1962. Some factors influencing survival, growth, and flowering of intraspecific and interspecific pine grafts. *J. For.* 60:785-789.
- Bleymuller, H. 1976. Investigations on the dependence of flowering in spruce (*Picea abies* (L.) Karst.) upon age and hormone treatment. *Silvae Genetica* 25(2):83-85.
- Bonnet-Masimbert, M. 1982. Effect of growth regulators, girdling, and mulching on flowering of young European and Japanese larches under field conditions. *Can. J. For. Res.* 12:270-279.
- Bramlett, D. L. and Belanger, R. P. 1976. Fertilizer and phenotypic selection increase growth and flowering of young Virginia pine. *For. Sci.* 22:461-467.
- Brix, H. and Portlock, F. T. 1982. Flowering response of western hemlock seedlings to gibberellin and water-stress treatments. *Can. J. For. Res.* 12:76-82.
- Cecich, R. A. 1983. Flowering in a jack pine seedling seed orchard increased by spraying with gibberellin A_{4/7}. *Can. J. For. Res.* 13:1056-1062.
- Cecich, R. A. 1985. White spruce (*Picea glauca*) flowering in response to spray application of gibberellin A_{4/7}. *Can. J. For. Res.* 15:170-174.
- Chalupka, W. 1978. Effect of growth regulators on the flowering of Scots pine (*Pinus sylvestris* L.) grafts. *Silvae Genetica* 27(2):62-65.
- Chalupka, W. 1980. Regulation of flowering in Scots pine (*Pinus sylvestris* L.) grafts by gibberellins. *Silvae Genetica* 29:118-121.
- Chalupka, W. 1981. Influence of growth regulators and polyethylene covers on flowering of Scots pine and Norway spruce grafts. *Silvae Genetica* 30(4/5):142-146.

- Chalupka, W. and Giertych, M. 1977. The effect of polyethylene covers on the flowering of Norway spruce (*Picea abies* (L.) Karst.) grafts. Arboretum Kornickie 22:185-192.
- Chalupka, W., Giertych, M. and Kopcewicz, J. 1982. Effect of polyethylene covers, a flower inducing treatment, on the content of endogenous gibberellin-like substances in grafts on Norway spruce. *Physiol. Plant.* 54:79-82.
- Dunberg, A. 1980. Stimulation of flowering in *Picea abies* by gibberellins. *Silvae Genetica* 29(2):51-53.
- Dunberg, A., Malmberg, G., Sassa, T. and Pharis, R. P. 1983. Metabolism of tritiated gibberellins A₄ and A₉ in Norway spruce, *Picea abies* (L.) Karst. *Plant. Physiol.* 71:257-262.
- Ebell, L. F. 1971. Physiology and biochemistry of flowering in Douglas-fir. In *Proc. of the IUFRO Meeting, Section 22, Working Group on Sexual Reproduction of Forest Trees. I. May 1970, Varperanta, Finland.*
- Greenwood, M. S. 1978. Flowering induced on young loblolly pine grafts by out-of-phase dormancy. *Science* 201:443-444.
- Greenwood, M. S. 1980. Reproductive development in loblolly pine: I. The early development of male and female strobili in relation to the long shoot growth behavior. *Amer. J. Bot.* 67(10):1414-1422.
- Greenwood, M. S. 1981. Reproductive development in loblolly pine. II. The effect of age, gibberellin plus water stress and out-of-phase dormancy on long shoot growth behavior. *Amer. J. Bot.* 68(9):1184-1190.
- Greenwood, M. S. 1982. Rate, timing, and mode of gibberellin application for female strobilus production by grafted loblolly pine. *Can. J. For. Res.* 12(4):998-1002.
- Greenwood, M. S., O'Gwynn, C. H. and Wallace, P. G. 1979. Management of an indoor, potted loblolly pine breeding orchard. In: *Proc. 15th South Forest. Tree Imp. Conf. Miss. State Univ.* pp. 94-98.
- Gregory, W. M., Guinness, W. M. and Davey, C. B. 1982. Fertilization and irrigation stimulate flowering and cone production in a loblolly pine seed orchard. *Southern Journal of Applied Forestry* 6:44-48.

- Hanover, J. W. 1976. Accelerated-optimal-growth: A new concept in tree production. *American Nurseryman* 144(10):12.
- Hanover, J. W. 1977. The performance, potential and precautions of accelerated-optimal-growth plants. *American Nurseryman* 145(9):12.
- Hanover, J. W. 1980. Control of tree growth. *Bioscience* 30:756-762.
- Hanover, J. W. and Bongarten, B. 1977. Accelerated-optimal growth: Applications in tree improvement. In *Proc. 25th Northeastern For. Tree Imp. Conf.* pp. 1-15.
- Hanover, J. W. and Reicosky, D. A. 1972. Accelerated growth for early testing of spruce seedlings. *For. Sci.* 18:92-94.
- Hanover, J. W., Young, E., Lemmien, W. A. and Van Slooten, M. 1976. Accelerated-optimal-growth: A new concept in tree production. Michigan State University Agr. Exp. Sta. East Lansing., Res. Rep. 317. 16 pp.
- Hare, R. C. 1978. Promoting flowering in loblolly and slash pine with branch, bud, and fertilizer treatments. In *Proc. Flowering and Seed Development in Trees: a Symposium.* Mississippi State University. F. Bonner (ed.) pp. 112-121.
- Hare, R. C. 1984. Application method and timing of gibberellin A₄/7 treatments for increasing pollen cone bud production in southern pines. *Can. J. For. Res.* 14:128-131.
- Hare, R. C., Snyder, E. B. and Schmidtling, R. C. 1977. Longleaf pine flowering in response to nitrogen fertilization, branch girdling, growth substances, and cultivation. In *Proc. Thirteenth Lake States For. Tree Imp. Conf., Univ. of Minnesota, St. Paul, MN.* pp 11-16.
- Harrison, D. L. and Owens, J. N. 1983. Bud development in *Picea engelmannii*. I. Vegetative bud development, differentiation, and early development of reproductive buds. *Can. J. Bot.* 61:2291-2301.
- Heidmann, L. J., Rietveld, W. J. and Trujillo, D. P. 1978. Fertilization increases cone production and diameter growth of a 55-year-old ponderosa pine stand in Arizona. In *Proc. Flowering and Seed*

- Development in Trees: A Symposium. Mississippi State University.
F. Bonner (ed.). pp. 197-205.
- Heimbürger, C. C. and Fowler, D. P. 1969. Precocious flowering in some pines of *Lariciones* group. *Silvae Genetica* 18:146-150.
- Heitmüller, H. H. and Melchior, G. 1960. *Silvae Genetica* 9:65-72. (as cited by Matthews 1963).
- Ho, R. H. 1985. Cone induction in white pine and Norway spruce. In Proc. 20th meeting of Canadian Tree Improvement Association.
- Karki, L. 1976. Flower induction hall for forest trees. Paper of The Foundation for Forest Breeding, Finland. 10 pp.
- Karki, L. 1979. Breeding the spruce genus (*Picea*) in a plastic greenhouse. The Foundation for Forest Tree Breeding in Finland. Info 3/1979. 3 pp.
- Kleinschmit, J., Schmidt, J. and Hosenfeld, B. 1980. Flower induction in spruce and Douglas-fir. *Forst Und Holzwirte*. 35(12):240-242.
- Lepistö, M. 1973. Accelerated birch breeding--in plastic greenhouses. *For. Chron.* pp. 172-173.
- Luukkanen, O. and Johansson, S. 1980. Effect of exogenous gibberellins on flowering in *Pinus sylvestris* grafts. *Physiol. Plant.* 50:365-370.
- Marquard, R. D. and Hanover, J. W. 1984a. Relationship between gibberellin A₄/7 concentration, time of treatment, and crown position on flowering of *Picea glauca*. *Can. J. For. Res.* 14:547-553.
- Marquard, R. D. and Hanover, J. W. 1984b. Sexual zonation in the crown of *Picea glauca* and flowering response to exogenous GA₄/7. *Can. J. For. Res.* 14:27-30.
- Marquard, R. D. and Hanover, J. W. 1985. Floral response of *Picea glauca* to gibberellin A₄/7, naphthaleneacetic acid, root-pruning, and biennial treatment. *Can. J. For. Res.* 15:743-746.
- Masters, C. J. 1981. Weyerhaeuser's seed orchard program. In Proc. Eighteenth Meeting of the Can. Tree Imp. Assoc., Duncan, B. C. Edited by C. W. Yeatman. pp 60 - 70.

- Masters, C. J., Meier, R. D., Skadsen, R. W. and Ross, S. D. 1981. The effects of rootpruning and girdling on flowering and seed set of Douglas-fir grafts. Weyer. Forest Res. Rep. (as cited by Masters 1981).
- Matthews, J. D. 1963. Factors affecting the production of seed by forest trees. Forestry Abstracts. 24(1). pp i-xiii.
- O'Gwynn, C. H. 1983a. Female flower stimulation in a greenhouse environment. In A Short Course on Accelerated Breeding, Weir R. (ed.), N. C. State University, School of Forest Resources.
- O'Gwynn, C. H. 1983b. Male flower stimulation through out-of-phase dormancy. In A Short Course on Accelerated Breeding, R. Weir (ed.), N. C. State University, School of Forest Resources.
- Olsen, H. C. 1978. Induction of flowering in Norway spruce. Forstl. Forsogsvaes. Dan. 36:231-265. As cited by Ross (1985).
- Owens, J. N. and Blake, M. D. 1985. Forest tree seed production. Can. For. Serv., Petawawa Nat. For. Inst. Info. Rep. PI-X-53. 161 pp.
- Owens, J. N. and Molder, M. 1974. Bud development in western hemlock. II. Initiation and early development of pollen cones and seed cones. Can. J. Bot. 52:283-294.
- Owens, J. N. and Molder, M. 1979. Bud development in *Larix occidentalis* II. Cone initiation and early development. Can. J. Bot. 57:1557-1572.
- Pharis, R. P. 1975. Promotion of flowering in conifers by gibberellins. For. Chron. 15:244-248.
- Pharis, R. P. 1976. Manipulation of flowering in conifers through the use of plant hormones. In Modern Methods of Forest Genetics. J. Miksche (ed.). Springer-Verlag. Berlin, Heidelberg, New York. pp. 265-282.
- Pharis, R. P. 1977. Promotion of flowering in the *Pinaceae* by hormones--a reality. In Proc. Thirteenth Lake States For. Tree Imp. Conf., Univ. of Minnesota, St. Paul, MN. pp. 1-10.

- Pharis, R. P. and Kuo, C. G. 1977. Physiology of gibberellins in conifers. *Can. J. For. Res.* 7:299-325.
- Pharis, R. P., Ross, S. D. and McMullan, E. 1980. Promotion of flowering in the *Pinaceae* by gibberellin. *Physiol. Plant.* 50:119-126.
- Philipson, J. J. 1985. The promotion of flowering in large field-grown Sitka spruce by girdling and stem injections of gibberellin A₄/7. *Can. J. For. Res.* 15:166-170.
- Pollard, D. F. W. and Portlock, F. T. 1984. The effects of photoperiod and temperature on gibberellin A₄/7 induced strobilus production of western hemlock. *Can. J. For.* 14:291-294.
- Puritch, G. S. and McMullan, E. E. 1981. A cut-branch technique for introducing solutions into conifers. *Can. J. For. Res.* 11:218-222.
- Ross, S. D. 1983. Enhancement of shoot elongation in Douglas-fir by gibberellin A₄/7 and its relation to the hormonal promotion of flowering. *Can. J. For. Res.* 13:986-994.
- Ross, S. D. 1985. Promotion of flowering in potted *Picea engelmannii* (Perry) grafts: effects of heat, drought, gibberellin A₄/7, and their timing. *Can. J. For. Res.* 15:618-624.
- Ross, S. D. and Greenwood, M. S. 1979. Promotion of flowering in the *Pinaceae* by gibberellins. II. Grafts of mature and immature *Pinus taeda*. *Physiol. Plant.* 45:207-210.
- Ross, S. D. and Pharis, R. P. 1982. Recent developments in enhancement of seed production in conifers. In *Proc. Eighteenth Meeting of the Can. Tree Imp. Assoc.*, Duncan, B. C., D. F. W. Pollard, D. G. Edwards, and C. W. Yeatman (eds.). pp. 26-38.
- Ross, S. D., Eastham, A. M. and Bower, R. C. 1985a. Potential for indoor-potted seed orchards. In *Symposium on Conifer Tree Seed in the Inland Mountain West*. Univ. of Montana. 5-6 August.

- Ross, S. D., Pharis, R. P. and Binder, W. D. 1983. Growth regulators and conifers: their physiology and potential uses in forestry. In Plant Growth Regulating Chemicals, Volume II, L.G. Nickell (ed.), CRC Press, Inc., Boca Raton, FL. pp. 35-78.
- Ross, S. D., Pharis, R. P. and Heaman, J. C. 1980. Promotion of cone and seed production in grafted and seedling Douglas-fir seed orchard by application of gibberellin A₄/7 mixture. Can. J. For. Res. 10:464.
- Ross, S. D., Piesch, R. F. and Portlock, F. T. 1981. Promotion of cone and seed production in rooted ramets and seedlings of western hemlock by gibberellins and adjunct cultural treatments. Can. J. For. Res. 11:90-98.
- Ross, S. D., Webber, J. E., Pharis, R. P. and Owens, J. N. 1985b. Interaction between gibberellin A₄/7 and root-pruning on the reproductive and vegetative process in Douglas-fir. I. Effects on flowering. Can. J. For. Res. 15:341-347.
- Schmidtling, R. C. 1974. Fruitfulness in conifers: nitrogen, carbohydrate, and genetic control. In Proc. Third North Am. For. Biol. Workshop. pp. 148-164.
- Schmidtling, R. C. 1975. Fertilizer timing and formulation affect flowering in a loblolly pine seed orchard. In Proc. Thirteenth South. For. Tree Improv. Conf. pp. 153-160.
- Schmidtling, R. C. 1981. The inheritance of precocity and its relationship with growth in loblolly pines. Silvae Genetica 30(6):188-192.
- Schmidtling, R. C. 1983. Rootstock influences flowering, growth, and survival of loblolly pine grafts. For. Sci. 29:117-124.
- Simak, M. 1978. Precocious flower induction in *Pinus sylvestris* by grafting. In International Symposium on "Flowering and Seed Development in Trees". Starkville, MS, USA, May 15-18, 1978.
- Sprague, J., Jett, J. B. and Zobel, B. 1978. The management of southern pine seed orchards to increase seed production. In Proc. Flowering and Seed Development in Trees: A Symposium. Mississippi State University. F. Bonner (ed.). pp. 145-16

- Steinbrenner, E. C., Duffield, J. W. and Campbell, R. K. 1960. Increased cone production of young Douglas-fir following nitrogen and phosphorous fertilization. *J. For.* 58:105-110. As cited by Sprague et al. 1978.
- Summerville, K. O., Jett, J. B. and Davey, C. B. 1978. Conelet abortion in a longleaf seed orchard: fertilization and irrigation effects. In *Proc. Flowering and Seed Development in Trees: A Symposium*. Mississippi State University. F. Bonner (ed.). pp. 187-196.
- Sweet, G. B. 1975. Flowering and seed production. In *Seed Orchards*. Forestry Commission Bulletin No. 54. 149 pp. Faulkner, R. (ed.). pp. 72-82.
- Tompsett, P. B. and Fletcher, A. M. 1977. Increased flowering of Sitka spruce (*Picea sitchensis* (Bong.)Carr.) in a polythene house. *Silvae Genetica* 26(2/3):84-85.
- Tompsett, P. B. and Fletcher, A. M. 1979. Promotion of flowering on mature *Picea sitchensis* by gibberellin and environmental treatments. The influence of timing and hormonal concentration. *Physiol. Plant.* 45:112-116.
- Webber, J. E., Ross, S. D., Pharis, R. P. and Owens, J. N. 1985. Interaction between gibberellin A₄/7 and root-pruning on the reproductive and vegetative process in Douglas-fir. II. Effects on shoot elongation and its relationship to flowering. *Can. J. For. Res.* 15:348-353.
- Weir, B. 1983. Alternatives to the greenhouse methods of flower stimulation. In *A Short Course on Accelerated Breeding*, R. Weir (ed.), N.C. State University, School of Forest Resources.
- Wheeler, N. C. 1979. Effect of continuous photoperiod on growth and development of lodgepole pine seedlings and grafts. *Can. J. For. Res.* 9:276-283.

- Wheeler, N. C., Wample, R. L. and Pharis, R. P. 1980. Promotion of flowering in the *Pinaceae* by gibberellins. IV. Seedlings and sexually mature grafts of lodgepole pine. *Physiol. Plant.* 50:340-346.
- Wheeler, N. C., Ying, C. C. and Murphy, J. C. 1982. Effect of accelerating growth on flowering in lodgepole pine seedlings and grafts. *Can. J. For. Res.* 12:533-537.
- Wheeler, N. C., Masters, C. J., Cade, S. C., Ross, S. D., Keeley, J. W. and Hsin, L. Y. 1985. Girdling: an effective and practical treatment for enhancing seed yields in Douglas-fir seed orchards. *Can. J. For. Res.* 15:505-510.
- Young, E. and Hanover, J. W. 1976. Accelerating maturity in *Picea* seedlings. *Acta Horticulture* 56:105-114.

